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Sustained Inhibition of Proliferative Response After Transient FGF Stimulation Is Mediated by Interleukin 1 Signaling

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Transient FGF stimulation of various cell types results in FGF memory—a sustained blockage of efficient proliferative response to FGF and other growth factors. FGF memory establishment requires HDAC activity, indicating its epigenetic character. FGF treatment stimulates proinflammatory NF κ B signaling, which is also critical for FGF memory formation. The search for FGF-induced mediators of FGF memory revealed that FGF stimulates HDAC-dependent expression of the inflammatory cytokine IL1 α . Similarly to FGF, transient cell treatment with recombinant IL1 α inhibits the proliferative response to further FGF and EGF stimulation, but does not prevent FGF receptor-mediated signaling. Interestingly, like cells pretreated with FGF1, cells pretreated with IL1 α exhibit enhanced restructuring of actin cytoskeleton and increased migration in response to FGF stimulation. IRAP, a specific inhibitor of IL1 receptor, and a neutralizing anti-IL1 α antibody prevent the formation of FGF memory and rescue an efficient proliferative response to FGF restimulation. A similar effect results following treatment with the anti-inflammatory agents aspirin and dexamethasone. Thus, FGF memory is mediated by proinflammatory IL1 signaling. It may play a role in the limitation of proliferative response to tissue damage and prevention of wound-induced hyperplasia.

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We recently determined that transient FGF stimulation results in a stable inhibition of proliferative response to repeated FGF treatment (Poole et al., 2014). This phenomenon termed “FGF memory” has been detected in fibroblasts, endothelial cells, mesenchymal stem cells, and adipose-derived stem cells. Cell stress occurring in damaged tissues can result in the nonclassical release of FGF1 (Prudovsky et al., 2008). We hypothesized that FGF memory may serve to moderate the proliferative response after tissue damage, and thus prevent hyperplasia and decrease scar formation. Establishment of FGF memory depends on NF κ B signaling and requires histone deacetylase (HDAC) activity (Poole et al., 2014). Based on these results, a study was undertaken to understand the molecular mechanisms underlying FGF memory. We found that FGF enhances the expression of interleukin (IL) 1 α , and this effect depends on HDAC activity. Similarly to FGF, transient treatment with IL1 α drastically inhibits the proliferative response to subsequent stimulation with FGF but does not interfere with FGF-promoted signaling and cell migration. Moreover, cell pretreatment with both FGF1 and IL1 α blocks the proliferative response to an unrelated growth factor, EGF. FGF memory is abolished by IRAP, the competitive inhibitor of IL1 receptor type I, and a specific neutralizing antibody against IL1 α . In addition, anti-inflammatory agents aspirin and dexamethasone eliminate it. Collectively, these results show that FGF memory is mediated by IL1 α production resulting in enhanced inflammatory signaling.

Materials and Methods

Cell cultures

Swiss 3T3 (ATCC, Manassas, VA) cells and mouse lung endothelial cells Le II (Friesel and Maciag, 1988) were maintained in DMEM

(HyClone, Logan, UT) supplemented with 10% bovine calf serum (HyClone) and 1% antibiotic-antimycotic mixture (GIBCO, Grand Island, NY). Quiescence was induced by culturing cells in DMEM containing 0.2% bovine calf serum and 5 units/mL heparin (Sigma, St. Louis, MO).

Cell treatment

Prior to stimulation, cells were transferred for 48 h to quiescence (Q) medium: DMEM with 5 units/mL heparin and 0.2% bovine calf serum. This basic medium was used in all types of stimulation. Upon induction of quiescence as well as withdrawal of growth factors, the cells were washed twice with DMEM medium containing 5 units/mL heparin.

Recombinant human FGF1 was prepared as described (Forough et al., 1991) and applied at 10 ng/mL. Human recombinant EGF (Lonza, Portsmouth, NH) and IL1 α (Roche, Nutley, NJ) were used

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at 10 ng/mL. Human recombinant IRAP (Roche) and neutralizing antibodies against mouse IL1 α (R&D, Minneapolis, MN) were used at 600 or 300 ng/mL.

The following chemical compounds were used: aspirin (acetylsalicylic acid) (Sigma), dexamethasone (Sigma), trichostatin A (TSA) (Sigma) and panabinoestat (Selleck, Houston, TX).

Dna synthesis study

Throughout the final 36 h of each stimulation condition, the cells were exposed to 10 μ g/mL bromodeoxyuridine (BrdU) (Sigma). Once stimulation schedules were completed, the cells were fixed for a minimum of 10 min in 100% ethanol, washed with PBS, and DNA was denatured by incubation in 1 N HCl at 55°C for 30 min. The residual acid was then washed with PBS. Non-specific binding of antibodies was prevented by a 30 min pre-exposure to blocking buffer (5% bovine albumin, 0.1% Triton X-100, 0.1% sodium azide in PBS), followed by an 1 h incubation in a 1:500 dilution of monoclonal mouse anti-BrdU antibody (Dako, Carpinteria, CA) in blocking buffer. The cells were then washed with PBS and incubated for 30 min in 1:500 dilution of Alexa 546-conjugated anti-mouse IgG antibodies (Invitrogen). Counting of BrdU-positive nuclei in cell populations was performed using an Olympus IX70 microscope with a combination of fluorescence and phase contrast. Two coverslips were studied per each experimental point. For each coverslip, 500 nuclei were counted; the number of fluorescent nuclei was recorded to acquire BrdU incorporation percentages.

Cell migration assay

Linear scratches in cell monolayers were made using a 1 mL pipette tip. Photographs of scratches were taken at 0 and 24 h after monolayer wounding. Per each experimental condition, three independent wells were studied, and in each of them fifteen microscopic fields were photographed using the 10 X objective. The mean distances covered by the migrating fronts of monolayers and corresponding SEM were calculated.

Confocal fluorescence microscopy

The effects of FGF stimulation on the actin skeleton of naïve and IL1 α -pretreated Swiss 3T3 cells were studied using confocal fluorescence microscopy. The cells were fixed with 4% neutral formalin, pre-incubated in blocking permeabilizing buffer (PBS with 5% BSA and 0.1% Triton X100), and then stained with Oregon green-conjugated phalloidin and TOPRO3 (both from Invitrogen, Carlsbad, CA). Cell images were taken using Leica SP8 confocal microscope at the MMCRI confocal microscopy facility.

Immunoblot analysis

Lysates were prepared from cell monolayers after various schedules involving IL1 α and FGF1 stimulation. The cells were washed and scraped in ice-chilled PBS and centrifuged for collection at 2,500 rpm for 10 min. NPB buffer (20 mM Tris-HCl (pH 7.4), 250 mM sucrose, 60 mM KCl, 20 mM EDTA, 1.5 M NaCl, 1% TritonX-100, 0.1% deoxycholic acid, and a 1:50 dilution of protease inhibitor cocktail from Sigma) was used to lyse the cells. Relative protein concentrations were determined with Coomassie Plus Protein Assay Kit (Pierce, Rockford, IL) using a DU 640 spectrophotometer (Beckman, Fullerton, CA) at an excitation wavelength of 595 nm. The lysate was then mixed with an equal volume of SDS-PAGE sample buffer and incubated at 95°C for 10 min. Equivalent sample amounts were resolved by 12% PAGE and transferred to membranes Hybond-P (GE Healthcare, Little Chalfont, UK). The membranes were blocked in 5% fat-free dry

milk diluted in TBS-Tween buffer at 42°C for 2 h, and then blotted with the appropriate primary mouse or rabbit antibody overnight at 4°C.

Membranes blotted with mouse anti- β -actin antibodies (Sigma) served as loading controls. Antibodies against the following proteins were used: rabbit antibodies against phosphorylated Erk1/2 (Sigma) and against cyclin D1 (Santa Cruz Biotechnologies, Santa Cruz, CA), and mouse monoclonal antibodies recognizing cyclin A (Millipore, Temecula, CA). The bound primary antibodies were visualized using horseradish peroxidase-conjugated goat antibodies against rabbit or mouse IgG (BioRad, Hercules, CA) and the ECL detection system (Amersham, Piscataway, NJ).

RT-PCR

RNA was prepared from Swiss 3T3 cells using the RNeasy kit (Qiagen, Hilden, Germany). Expression of IL1 β and IL1 α was assessed by RT-PCR using the SuperScript kit (Invitrogen). The following pairs of primers were utilized:

IL1 α : as. -5'-GTC TCA TGA AGT GAG CCA TAG C-3', s. -5'-CAA GAT GGC CAA AGT TCG TGA C-3'.

IL1 β : as. -5'-CAG GAC AGG TAT AGA TTC TTT CTT TT-3', s. -5'-ATG GCA ACT GTT CCT GAA CTC AAC T-3'.

β -actin: as. -5'-GTC TCA AAC ATG ATC TGG G-3', s. 5'-AGA AAA TCT GGC ACC ACA CC-3'.

For qRT-PCR, we used the same IL1 α primers and the following cyclophilin primers:

as. -5'-CAG TGC TCA GAG CTC GAA AG-3', s. -5'-CCA CCG TGT TCT TCG ACA T-3'.

Elisa

IL1 α concentration in cell lysates was determined using an ELISA kit from eBioscience (San Diego, CA) according to the instructions of the manufacturer.

Statistical analysis

Each experiment was repeated at least three times with consistent results. In the studies analyzing DNA synthesis, percentage of BrdU-labeled cells with 95% confidence interval was calculated for each condition. In qRT-PCR and ELISA studies of IL1 α expression and in the studies of cell growth and migration, Student's *t* test was used to assess the significance of observed effects.

Results

FGF induces the expression of IL1 α

The observations that FGF stimulation activates NF κ B signaling, and establishment of FGF memory depends on NF κ B pathway (Poole et al., 2014) indicated that FGF may induce the expression of a proinflammatory cytokine. Interestingly, IL1 α , a potent inducer of inflammation and activator of NF κ B signaling, represses cell proliferation (Ohmori et al., 1988; Ikeda et al., 1991) and plays a key role in some cases of cell senescence (Maier et al., 1990; McCarthy et al., 2013). We used RT-PCR to assess the effect of FGF1 stimulation on the expression of proinflammatory cytokines and inducers of NF κ B signaling, IL1 α , and IL1 β , in Swiss 3T3 cells (Fig. 1A). While IL1 β expression remained unchanged, FGF1 stimulated the expression of IL1 α . qRT-PCR study has shown a 20-fold induction of IL1 α by FGF (Fig. 1B). Interestingly, the induction of IL1 α expression by FGF1 was abolished by the HDAC inhibitor panabinoestat (Fig. 1C). The RT-PCR results were confirmed by determining the IL1 α content in the lysates of Swiss 3T3 cells stimulated with FGF1 in presence or absence of HDAC inhibitors (Fig. 1D). It is noteworthy that the

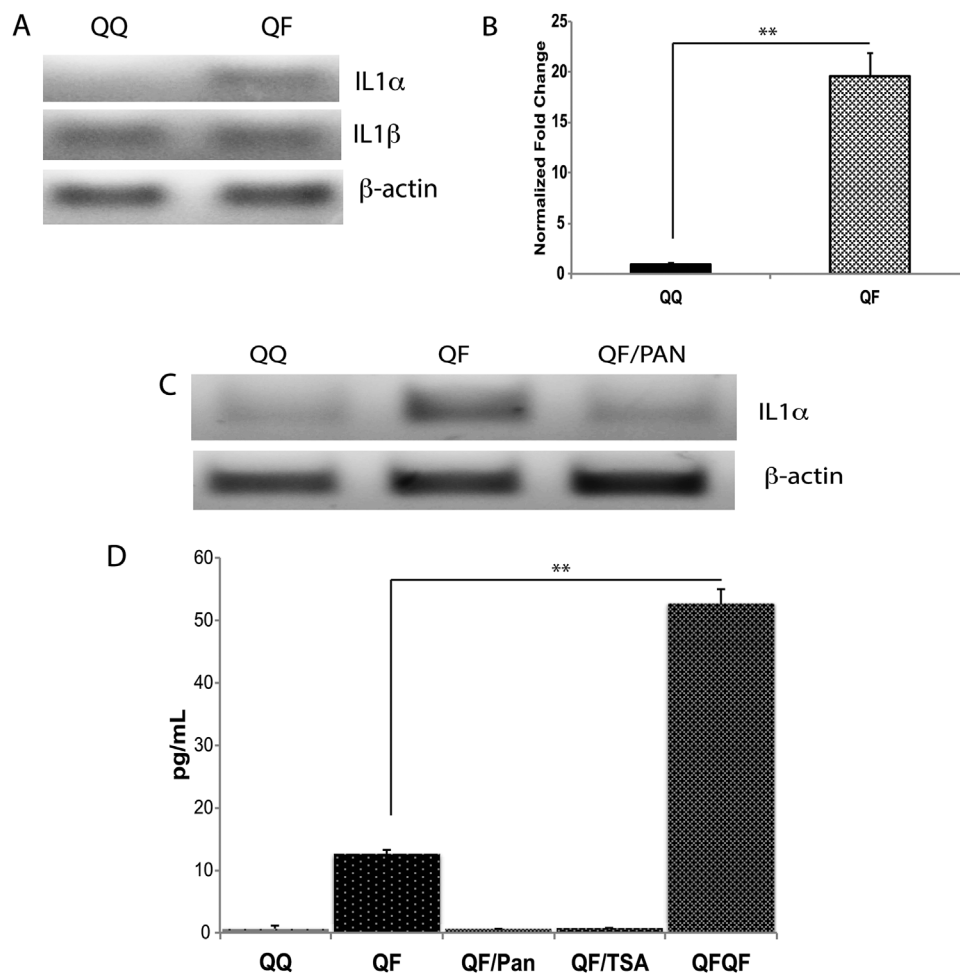


Fig. 1. FGF1 induces IL1 α expression in an HDAC-dependent manner. **A:** Confluent Swiss 3T3 cell cultures were incubated for 48 h in quiescence medium (0.2% serum) and then transferred for 36 h to quiescence medium with (QF) or without (QQ) 10 ng/mL FGF1. Expression of IL1 β and IL1 α was determined by RT-PCR. **B:** Cells were treated similarly to **A** and IL1 α expression was determined by qRT-PCR. **Indicates $P < 0.01$. **C:** Cells were treated similarly to **A** with an additional condition, when FGF stimulation was performed in the presence of 200 nM panabinostat (QF/PAN). Expression of IL1 α was determined by RT-PCR. **D:** Cells were treated similarly to **C** except that quiescence was extended to 168 h in QQ, and to 132 h in QF and QF/PAN, and two additional schedules were added: (i) repeated FGF1 stimulation (QFQF), when following the primary FGF1 stimulation cells were transferred for 48 h to quiescence medium and after that restimulated for 36 h with FGF1; (ii) FGF1 stimulation of naïve cells in presence of 300 nM trichostatin A (QF/TSA). Cell lysates were prepared and equalized based on total protein concentration. The concentration of IL1 α in the equalized lysates was determined using an ELISA kit. **Indicates $P < 0.01$.

concentration of IL1 α in the cell lysates after repeated FGF1 stimulation was approximately five times higher than after the primary stimulation. Thus, FGF induces the expression of IL1 α in an HDAC-dependent manner.

Transient pretreatment with IL1 α prevents proliferative response to FGF and EGF

Because FGF1 induces the expression of IL1 α , we assessed whether similar to transient FGF1 stimulation, transient application of IL1 α to Swiss 3T3 cells prevents proliferative response to further FGF stimulation. The following schedules of cell treatment were applied: QQ – 168 h of quiescence; QF – 132 h quiescence, 36 h FGF1; QIQQ – 48 h quiescence, 36 h IL1 α , 84 h quiescence; QIQF – 48 h quiescence, 36 h IL1 α , 48 h quiescence, 36 h FGF1. BrdU incorporation during the last

36 h of each schedule was determined (Fig. 2A). IL1 α pretreatment (QIQF) resulted in a 10-fold decrease of DNA synthesis in comparison to FGF1 stimulation of naïve quiescent cells (QF) (Fig. 2B). In a series of similar experiments, we found that transient FGF1 and IL1 α stimulations inhibited the proliferative response to epidermal growth factor (EGF) (Fig. 2C,D).

Transient pretreatment with IL1 α does not interfere with the activation of Erk1/2 and induction of cyclin D in response to FGF stimulation but prevents the induction of cyclin A

To understand the mechanisms through which the pretreatment of FGF1 inhibits growth factor-induced proliferation, we used immunoblotting to assess the

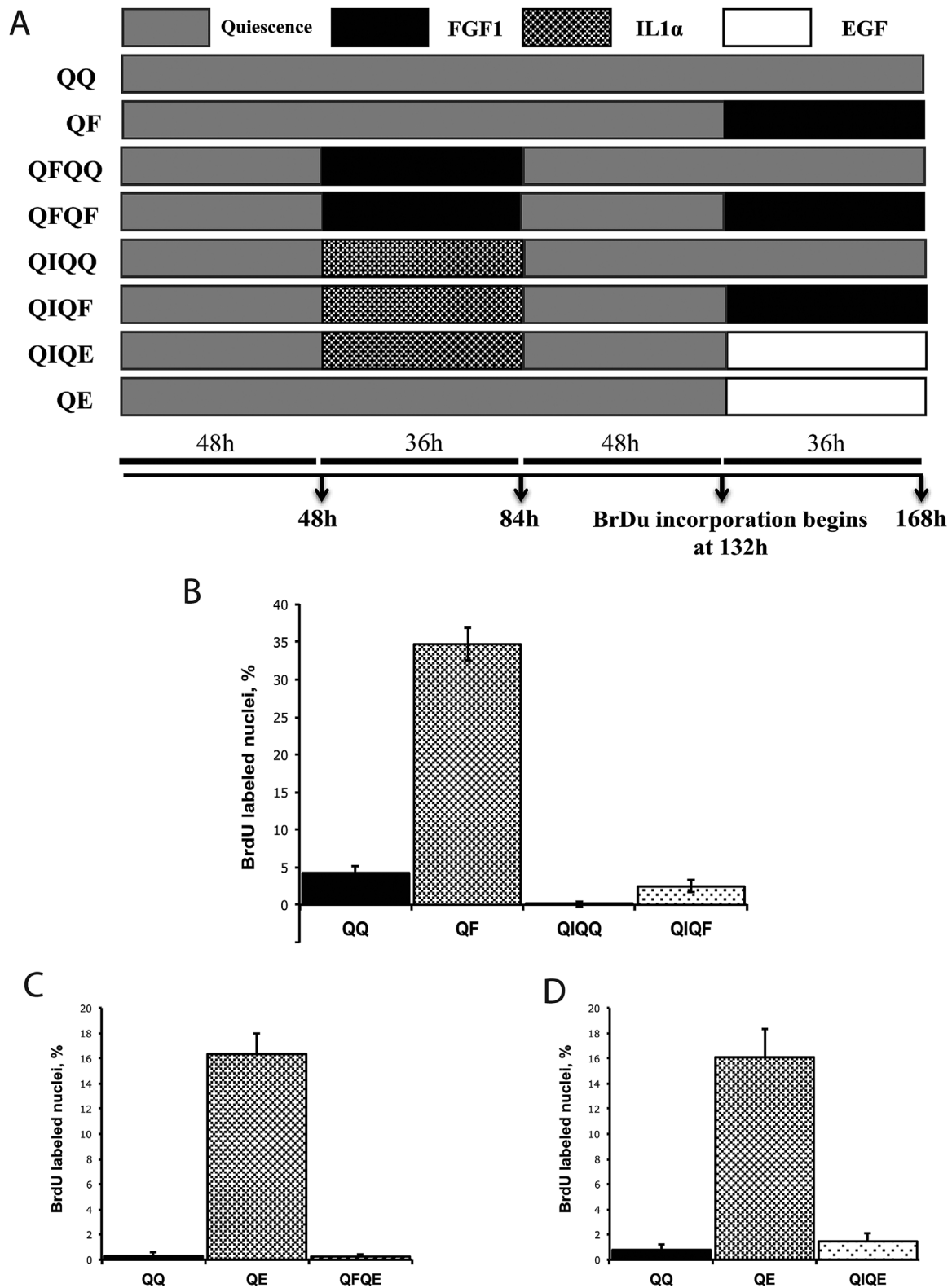


Fig. 2. Transient treatment with IL1 α results in a stable repression of proliferative response to growth factors. **A:** Schedules of cell stimulations with IL1 α , FGF1, and EGF (explanation in the text). BrdU labeling of Swiss 3T3 cells was performed during the final 36 h of each stimulation schedule. **B:** Acute repression of proliferative response to FGF1 (10 ng/mL) after transient pretreatment with IL1 α (10 ng/mL) and the intermediate 48 h period of quiescence. **C,D:** Transient pretreatment with FGF1 (**C**) or IL1 α (**D**) inhibits the proliferative response to EGF (10 ng/mL). In **B**, **C**, and **D**, the percentage of BrdU labeled cells with 95% confidence interval for each condition is shown. The experiments were repeated at least three times and the results of representative experiments are presented.

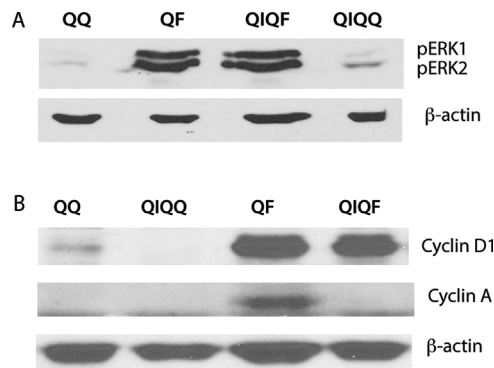


Fig. 3. FGF-induced proliferative signaling in Swiss 3T3 cells pretreated with IL1 α . Phosphorylation of Erk1/2 (A) and expression of cyclins D1 and A (B) in naïve (QF) and IL1 α -pretreated (QIQF) Swiss 3T3 cells. The duration of final FGF stimulation was 30 min (A) or 24 h (B). Total cell lysates were resolved by SDS-PAGE and immunoblotted for phospho-Erk1/2, cyclin D1, cyclin A, or β -actin.

signaling events and expression of key cell cycle proteins in response to FGF stimulation of IL1 α -pretreated (QIQF) and naïve (QF) Swiss 3T3 cells. Thirty minutes after final FGF stimulation, the phosphorylation of Erk1/2 reflecting the early signaling downstream of FGFR was identical in both QIQF and QF cells (Fig. 3A). The same was true for the expression of cyclin D1 24 h after stimulation, a period corresponding to late G1/early S phase after the exit from quiescence (Fig. 3B). In contrast, unlike naïve cultures, cells with IL1 α history failed to express cyclin A, the key regulator of the S-phase initiation and transition, in response to 24 h FGF stimulation (Fig. 3B).

IL1 α pretreatment enhances the restructuring of actin cytoskeleton and cell migration in response to FGF stimulation

Cells transiently treated with FGF respond to FGF restimulation by enhanced actin cytoskeleton reorganization and migration (Poole et al., 2014). We used phalloidin staining to compare the status of the actin cytoskeleton after FGF treatment of naïve (QF) and IL1 α -pretreated Swiss 3T3 (QIQF) cells. Confocal fluorescence study demonstrated a stronger formation of actin fibers in cells with the history of IL1 α treatment (Fig. 4A). In agreement with these results, the study of wounded monolayers showed that QIQF cells exhibit a faster migration than cells in QF populations (Fig. 4B).

FGF memory depends on IL1 receptor signaling

The induction of IL1 α expression by FGF and the similarity between the effects of FGF and IL1 α pretreatment on proliferative and migratory response to FGF stimulation indicate that FGF memory could be dependent on IL1 signaling. To assess this hypothesis, we determined the effect of IRAP, a specific competitive antagonist of IL1 receptor type I, on FGF memory. When IRAP was applied throughout the QFQF schedule of a typical FGF memory experiment, it completely rescued the proliferative response of Swiss 3T3 cells after repeated FGF stimulation (Fig. 5A).

Neutralization of IL1 α rescues the proliferative response to the secondary FGF stimulation

To assess the role of IL1 α in FGF memory, we evaluated the effect of a specific neutralizing anti-IL1 α antibody upon the proliferative response of Swiss 3T3 cells to secondary FGF stimulation. We found that DNA synthesis after repeated FGF treatment was rescued when anti-IL1 α antibodies were present throughout the QFQF experimental schedule (Fig. 5B).

Anti-inflammatory drugs rescue the proliferative response to the secondary FGF stimulation

IL1 α is a potent proinflammatory cytokine. To elucidate whether inflammatory signaling is required for FGF memory, we used two anti-inflammatory drugs: aspirin and dexamethasone. Both of them restored the proliferative response of Swiss 3T3 cells to the secondary FGF stimulation (Fig. 6A,B). Decreasing the concentrations of aspirin and IL1 α resulted in weaker rescuing effects (Supplementary Figure 1).

The rescuing effects of aspirin and dexamethasone on DNA synthesis were confirmed in FGF restimulation experiments using Le II mouse lung endothelial cells (Fig. 7A). Moreover, in special experiments where the time of secondary FGF stimulation was extended to 2 days, we detected a significant increase of LeII cell numbers upon the treatment with aspirin and dexamethasone (Fig. 7B,C).

Discussion

The earlier observation that transient stimulation with FGF results in a stable and potent inhibition of proliferative response to repeated growth factor stimulation (Poole et al., 2014) encouraged us to elucidate the key molecular determinant(s) of this phenomenon that had been termed FGF memory. The present study shows that IL1 α plays a critical role in FGF memory. The following results underlie this conclusion: (i) FGF treatment results in a HDAC-dependent IL1 α expression; (ii) IL1 α stimulation generates a cell phenotype similar to that observed in FGF-pretreated cells, i.e., strongly inhibited proliferative and enhanced migratory response to growth factor stimulation; (iii) IRAP and specific neutralizing anti-IL1 α antibodies inhibit FGF memory.

It has long been known that IL1 α exhibits anti-proliferative effects in non-transformed cells (Ohmori et al., 1988; Ikeda et al., 1991). In particular, it was found that senescence of human endothelial cells can be significantly delayed by the inhibition of IL1 α expression (Maier et al., 1990). In recent years, IL1 α was identified as a key determinant of the senescence-associated secretory cell phenotype, which is characterized by the inhibition of proliferation and production of several proinflammatory cytokines in addition to IL1 α (McCarthy et al., 2013).

We have earlier reported that transient FGF stimulation results in a sustained activation of NF κ B signaling, which is critical for FGF memory establishment (Poole et al., 2014). IL1 α (Niu et al., 2004; Melisi et al., 2009) and FGF (Muddasani et al., 2007; Salazar et al., 2014) have been demonstrated to activate the proinflammatory NF κ B pathway. Moreover, IL1 α expression and NF κ B signaling constitute an autoregulatory feedback loop that maintains IL1 α production (Niu et al., 2004). Proinflammatory signaling can result in the inhibition of DNA synthesis (Vlahos and Stewart, 1999). Concurrently, we have shown that the anti-inflammatory drugs, aspirin and dexamethasone, abolish IL1 α -mediated FGF memory. Thus, FGF-induced IL1 α expression and

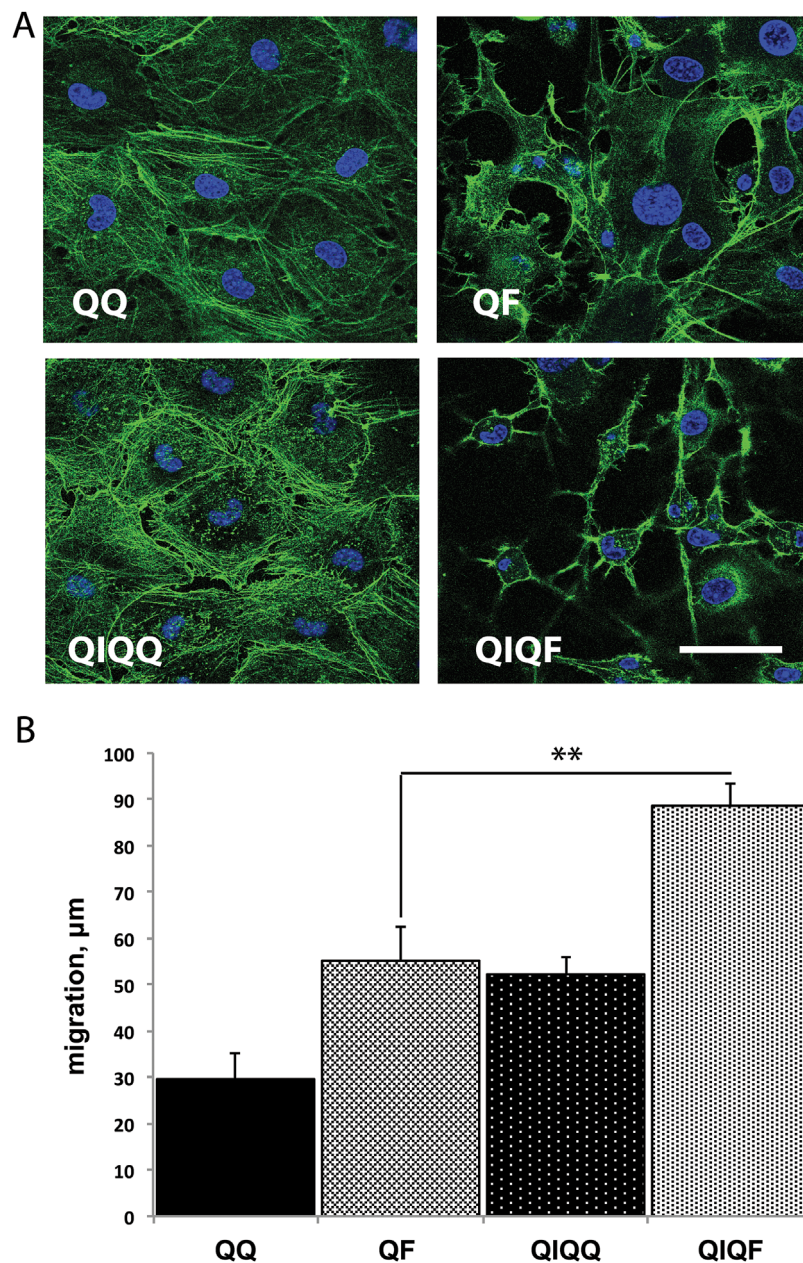


Fig. 4. Cell pretreated with IL1 α respond to FGF with an enhanced restructuring of the actin cytoskeleton and accelerated migration. **A:** IL1 α pretreatment results in an exaggerated restructuring of the actin cytoskeleton in response to FGF stimulation of Swiss 3T3 cells. Following formalin fixation (36 h after final FGF stimulation), cells were co-stained with Oregon green-phalloidin for F-actin (green) and DAPI for DNA (blue), and studied using a confocal microscope. Bar = 25 μ m. **B:** A standard experiment with FGF stimulation after IL1 α pretreatment was performed. At the end of the intermediate quiescence period, linear scratches in cell monolayers were made as described in Experimental Procedures and then the usual stimulation schedules were followed. Photographs of scratches were taken at 0 and 24 h after monolayer wounding. Mean distances (with corresponding SEM) covered by migrating monolayer fronts by 24 h are presented with **indicating $P < 0.01$.

proinflammatory signaling limit the proliferative response to continuous FGF stimulation. The experiments involving transient cell treatment with IL1 α demonstrated a sustained inhibition of proliferative response to FGF and EGF stimulation. Similar to earlier reported experiments with FGF restimulation (Poole et al., 2014), cells pretreated with IL1 α respond to FGF by Erk1/2 phosphorylation and cyclin

D1 expression. Moreover, their cytoskeletal and migratory responses to FGF are significantly stronger than in naïve cells. These results indicate that FGFR-mediated signaling, progression from quiescence to G1 phase as well as morphological and migratory changes are resistant to IL1 α or increased by it. Indeed, IL1 signaling enhances cell migration in the course of inflammation (Mitchell et al.,

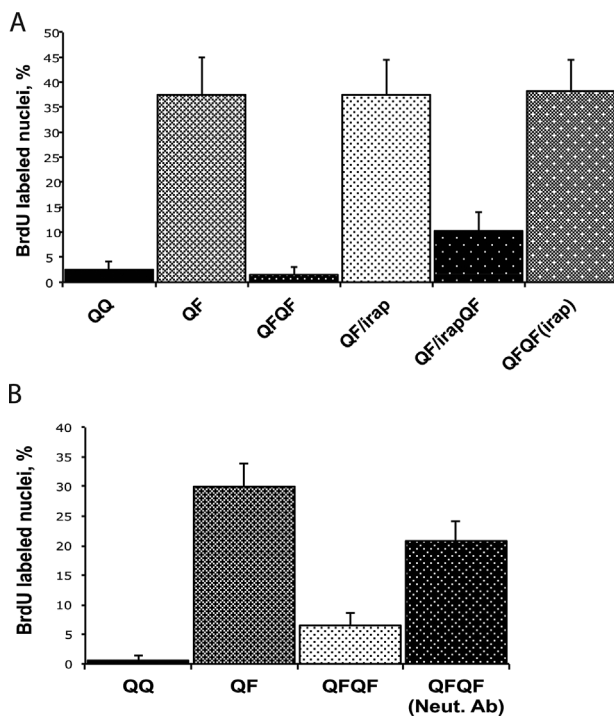


Fig. 5. Blockage of IL1 signaling inhibits FGF memory. A: IRAP, the inhibitor of IL1 receptor type I rescues the proliferative response of Swiss 3T3 cells to repeated FGF1 stimulation. QQ – quiescent cells, QF – naive cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules: (i) QF/irap – 600 ng IRAP present during the stimulation of naive cells; (ii) QF/irapQF – 600 ng/mL IRAP during the primary FGF1 stimulation; (iii) QFQF(irap) – 600 ng/mL IRAP present starting from the first FGF1 stimulation to the end of restimulation. B: Neutralizing antibodies against mouse IL1 α rescue the proliferation of Swiss 3T3 cells restimulated with FGF1. QFQF (neut. Ab) – 300 ng/mL neutralizing antibody present starting from the first FGF1 stimulation to the end of restimulation. In A and B, the percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. In each case, the experiments were triplicated, and the results of a representative experiment are shown.

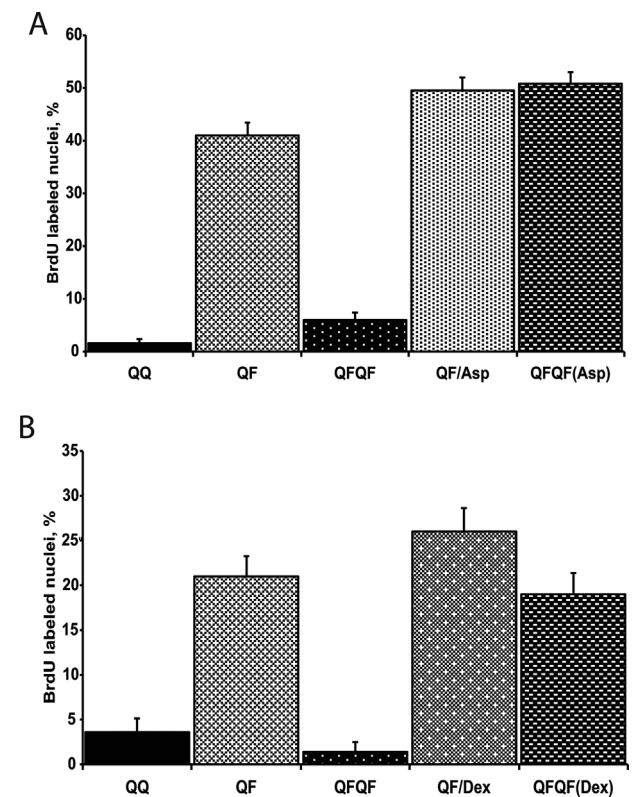


Fig. 6. Anti-inflammatory agents aspirin (A) and dexamethasone (B) suppress FGF memory in Swiss 3T3 cells. QQ – quiescent cells, QF – naive cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules in A: (i) QF/asp – 1 mM aspirin present during the FGF1 stimulation of naive cells; (ii) QFQF(asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation. Additional schedules in B: (i) QF/dex – 1 μ M dexamethasone present during the FGF1 stimulation of naive cells; (ii) QFQF(Dex) – 1 μ M dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. In A and B, the percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. In each case, the experiments were triplicated, and the results of a representative experiment are shown.

2007). On the other hand, FGF-induced IL1 α prevents DNA replication. The mechanisms of the sustained inhibition of proliferative response in cells transiently treated with IL1 α and FGF1 require further studies. The inability of such cells to express cyclin A in response to FGF indicates the blockage of cell cycle events immediately preceding the onset of the S phase.

FGF memory and induction of IL1 α expression require not only NF κ B signaling but also HDAC activity. Deacetylation of NF κ B p65 is required for its nuclear translocation and retention and thus for efficient NF κ B signaling (Grabiec et al., 2012; Liu and McCall, 2013; Ziesche et al., 2013; Zhang et al., 2015). FGF memory could be dependent on HDAC-mediated histone deacetylation or on HDAC-dependent activation of NF κ B signaling or on both. As we have reported (Poole et al., 2014), FGF does not change the expression of major HDACs. Thus, the search for a specific HDAC involved in FGF1 memory followed by the identification of its mechanism of action requires a comprehensive siRNA

knockdown screen study involving the assessment of FGF memory establishment, NF κ B signaling activity and IL1 α expression.

The significance of FGF memory for in vivo processes remains to be elucidated. This phenomenon could play a role in the regulation of the proliferative response during repair of tissue damage. It is noteworthy that liver regeneration after partial hepatectomy is accompanied by the increase of IL1 expression, which limits cell proliferation and prevents hyperplasia (Boulton et al., 1997; Sgroi et al., 2011). It is also interesting that endothelial-specific overexpression of FGF1 in transgenic mice paradoxically results not in the improvement of post-ischemic kidney repair, but in the inhibition of reparative processes and increased inflammation (Kirov et al., 2012). We hypothesize that FGF1 memory mediated by IL1 α production may regulate the repair of damaged tissues through the limitation of cell proliferation and enhancement of cell migration, which decrease scarring and help restore the normal tissue structure.

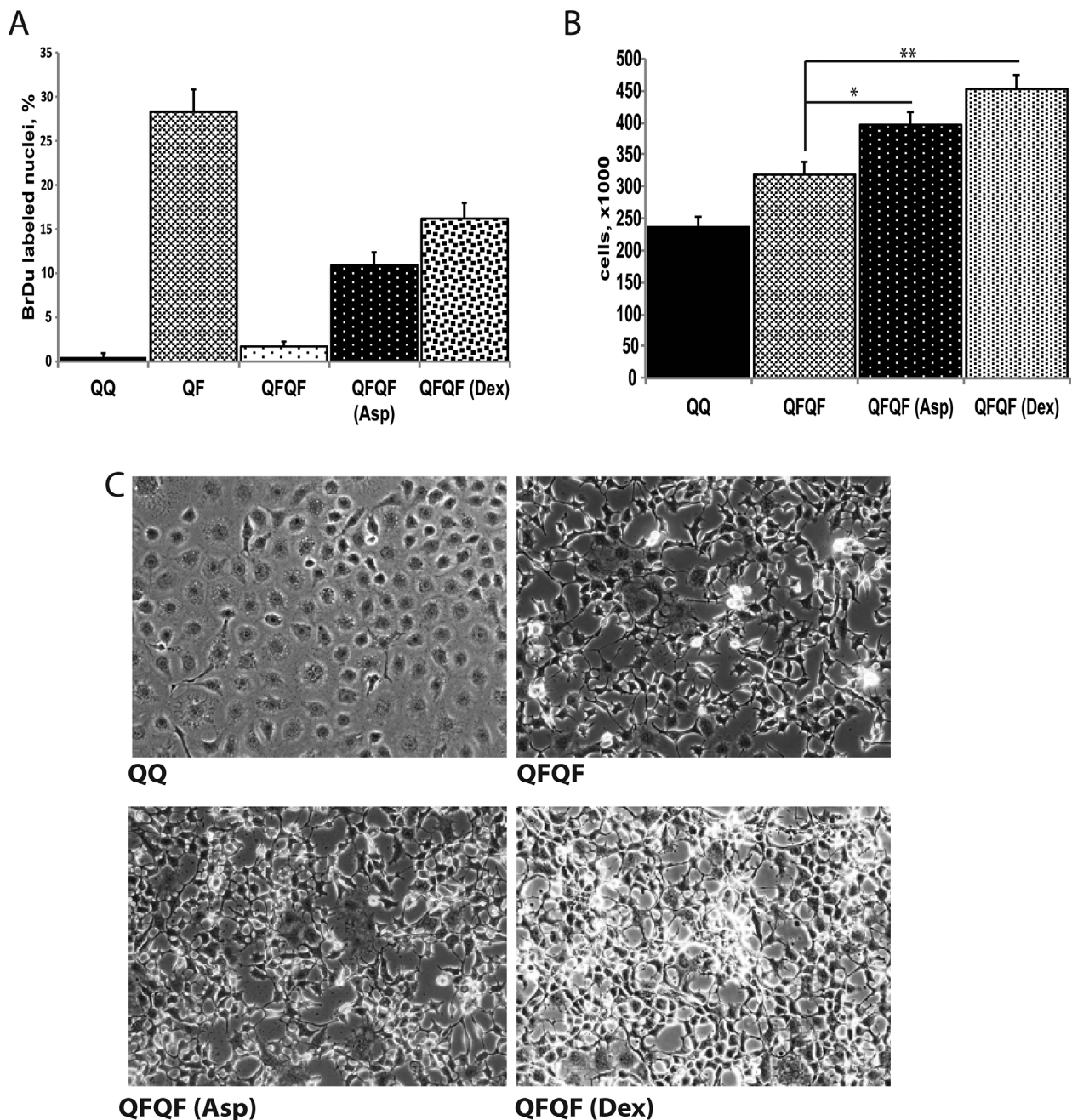


Fig. 7. FGF memory in endothelial cells is suppressed by anti-inflammatory agents. **A:** Aspirin and dexamethasone rescue DNA replication in Le II cells in response to FGF restimulation. QQ – quiescent cells, QF – naïve cells stimulated with FGF1, QFQF – cells restimulated with FGF1 (for details see Fig. 2A). Additional schedules: QFQF (Asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation; (ii) QFQF(Dex) – 1 μ M dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. The percentage of BrdU labeled cells with 95% confidence interval is shown for each condition. **B:** Aspirin and dexamethasone enhance the growth of Le II cells restimulated with FGF. The standard FGF restimulation experiment (see Fig. 2A) was modified by increasing the secondary FGF stimulation period to 48 h. At the end of the experiment, cells were trypsinized and cell numbers per well of 12-well plates were counted using hemocytometer. QQ – quiescent cells. QFQF – cells restimulated with FGF1. QFQF (Asp) – 1 mM aspirin present starting from the first FGF1 stimulation to the end of restimulation. QFQF(Dex) – 1 μ M dexamethasone present starting from the first FGF1 stimulation to the end of restimulation. *Indicates $P < 0.05$. **Indicates $P < 0.01$. **C:** Representatives phase contrast photographs of LeII cell growth under conditions described in B. Bar – 100 μ M.

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